

CLAIMS

1. A method of producing a polypeptide having hexose oxidase activity, comprising isolating or synthesizing a DNA fragment encoding the polypeptide, introducing said DNA fragment into
5 an appropriate host organism in which the DNA fragment is combined with an appropriate expression signal for the DNA fragment, cultivating the host organism under conditions leading to expression of the hexose oxidase active polypeptide and recovering the polypeptide from the cultivation medium or from the host organism.
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2. A method according to claim 1 wherein the DNA fragment is isolated from a marine algal species.
3. A method according to claim 2 wherein the marine algal species is one selected from the group consisting of *Chondrus crispus*, *Iridophycus flaccidum* and *Euthora cristata*.
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4. A method according to claim 1 wherein the host organism is a microorganism selected from the group consisting of a bacterial species, a fungal species and a yeast species.
5. A method according to claim 4 wherein the host organism is selected from the group consisting of *E. coli*, *Saccharomyces cerevisiae* and *Pichia pastoris*.
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6. A method according to claim 1 wherein the DNA fragment comprises at least one DNA sequence coding for an amino acid sequence selected from the group consisting of
- 25 (i) Tyr-Glu-Pro-Tyr-Gly-Gly-Val-Pro (SEQ ID NO:1),
- (ii) Ala-Ile-Ile-Asn-Val-Thr-Gly-Leu-Val-Glu-Ser-Gly-Tyr-Asp-X-X-X-Gly-Tyr-X-Val-Ser-Ser (SEQ ID NO:2),
- (iii) Asp-Leu-Pro-Met-Ser-Pro-Arg-Gly-Val-Ile-Ala-Ser-Asn-Leu-X-Phe (SEQ ID NO:3),

- (iv) Asp-Ser-Glu-Gly-Asn-Asp-Gly-Glu-Leu-Phe-X-Ala-His-Thr (SEQ ID NO:4),
- (v) Tyr-Tyr-Phe-Lys (SEQ ID NO:5),
- (vi) Asp-Pro-Gly-Tyr-Ile-Val-Ile-Asp-Val-Asn-Ala-Gly-Thr-X-Asp (SEQ ID NO:6),
- (vii) Leu-Gln-Tyr-Gln-Thr-Tyr-Trp-Gln-Glu-Glu-Asp (SEQ ID NO:7),
- (viii) X-Ile-Arg-Asp-Phe-Tyr-Glu-Glu-Met (SEQ ID NO:8),

where X represents an amino acid selected from the group consisting of Ala, Arg, Asn, Asp, Asx, Cys, Gln, Glu, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val,

and muteins and variants hereof.

7. A method according to claim 1 which comprises as a further step a purification of the polypeptide preparation initially recovered from the cultivation medium and/or the microorganisms to obtain a preparation in which the polypeptide is in a substantially pure form.

8. A method according to claim 1 wherein the polypeptide having hexose oxidase activity is a fusion product.

9. A polypeptide in isolated form having hexose oxidase activity, comprising at least one amino acid sequence selected from the group consisting of

- (i) Tyr-Glu-Pro-Tyr-Gly-Gly-Val-Pro (SEQ ID NO:1),
- (ii) Ala-Ile-Ile-Asn-Val-Thr-Gly-Leu-Val-Glu-Ser-Gly-Tyr-Asp-X-X-X-Gly-Tyr-X-Val-Ser-Ser (SEQ ID NO:2),

- 5 (iii) Asp-Leu-Pro-Met-Ser-Pro-Arg-Gly-Val-Ile-Ala-Ser-
Asn-Leu-X-Phe (SEQ ID NO:3),
- (iv) Asp-Ser-Glu-Gly-Asn-Asp-Gly-Glu-Leu-Phe-X-Ala-His-
Thr (SEQ ID NO:4),
- (v) Tyr-Tyr-Phe-Lys (SEQ ID NO:5),
- (vi) Asp-Pro-Gly-Tyr-Ile-Val-Ile-Asp-Val-Asn-Ala-Gly-
Thr-X-Asp (SEQ ID NO:6),
- (vii) Leu-Gln-Tyr-Gln-Thr-Tyr-Trp-Gln-Glu-Glu-Asp (SEQ
ID NO:7),
- 10 (viii) X-Ile-Arg-Asp-Phe-Tyr-Glu-Glu-Met (SEQ ID NO:8),

where X represents an amino acid selected from the group
consisting of Ala, Arg, Asn, Asp, Asx, Cys, Gln, Glu, Glx,
Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr
and Val,

15 and muteins and variants hereof.

10. A polypeptide according to claim 9 which is produced
according to the method of claim 1.

11. A polypeptide according to claim 9 which is produced by a
microbial cell selected from the group consisting of a bac-
20 terial cell, a fungal cell and a yeast cell.

12. A polypeptide according to claim 11 which is produced by
a cell selected from the group consisting of an *E. coli* cell,
a *Saccharomyces cerevisiae* cell and a *Pichia pastoris* cell.

13. A polypeptide according to claim 9 which is in a substan-
25 tially non-glycosylated form.

14. A polypeptide according to claim 9 which has functional characteristics identical or partially identical to those of hexose oxidase naturally occurring in *Chondrus crispus*.
15. A polypeptide according to claim 14 which when subjected to SDS-PAGE shows separate bands of 29, 40 and/or 60 kD.
16. A polypeptide according to claim 9 which shows an enzymatic activity at a pH in the range of 5-9.
17. A polypeptide according to claim 9 which has an optimum temperature for enzymatic activity being in the range of 20-60°C.
18. A polypeptide according to claim 9 which oxidizes at least one sugar selected from the group consisting of D-glucose, D-galactose, maltose, cellobiose, lactose, D-mannose, D-fucose and D-xylose.
19. A polypeptide according to claim 9 which has an isoelectric point in the range of 4-5.
20. A polypeptide according to claim 19 which has an isoelectric point of 4.3 ± 0.1 .
21. A polypeptide according to claim 19 which has an isoelectric point of 4.5 ± 0.1 .
22. A polypeptide according to claim 9 which is in a substantially purified form.
23. A polypeptide according to claim 9 which has a molecular weight as determined by gel filtration using Sephacryl S-200 Superfine (Pharmacia) which is in the range of 100-150 kD,
24. A polypeptide according to claim 23 which has an apparent molecular weight of $110 \text{ kD} \pm 10 \text{ kD}$.

25. A polypeptide according to claim 9 which is part of a fusion product comprising additional enzymatically active amino acid sequences.
26. A recombinant DNA molecule comprising a DNA fragment
5 coding for a polypeptide having hexose oxidase activity.
27. A DNA molecule according to claim 26 wherein the DNA fragment codes for a polypeptide comprising at least one amino acid sequence as defined in claim 9, or a mutein or derivative of such polypeptide.
- 10 28. A DNA molecule according to claim 27 comprising the DNA sequence (SEQ ID NO:30):

TGAATTCGTG GGTCTGAAGAG CCCTTTGCCT CGTCTCTCTG GTACCGTGTA TGTCAAAGGT 60
TCGCTTGAC ACTGAACTTC ACGATGGCTA CTCTTCCTCA GAAAGACCCC GGTTATATTG 120
TAATTGATGT CAACGCGGGC ACCGCGGACA AGCCGGACCC ACGTCTCCCC TCCATGAAGC 180
AGGGCTTCAA CCGCCGCTGG ATGGGAATA ATATCGATTT CGTTTATGTC GTGTACACTC 240
CTCAAGGTGC TTGTACTGCA CTGAGAGTG CTATGGAAAA GTGTTCTCCC GGTACAGTCA 300
GGATCGTCTC TGGCGGCCAT TGCTACGAGG ACTTCGTATT TGACGAATGC GTCAAGGCCA 360
TCATCAACGT CACTGGTCTC GTTGAAGTG GTTATGACGA CGATAGGGGT TACTTCGTCA 420
GCAGTGGAAGA TACAAATTGG GGCTCCCTCA AGACCTTGTT CAGAGACCAC GGAAGAGTTC 480
TTCCCGGGGG TTCCTGCTAC TCCGTCGCC TCGGTGGCCA CATTGTCCGC GGAGGTGACG 540
GCATTTTGGC CCGCTTGCAT GGCCTCCCCG TCGATTGGCT CAGCGGCGTG GAGGTCTGTCG 600
TTAAGCCAGT CCTCACCAGG GACTCGGTAC TCAAGTATGT GCACAAAGAT TCCGAAGGCA 660
ACGACGGGGA GCTCTTTTGG GCACACACAG GTGGCGGTGG CGGAACTTT GGAATCATCA 720
CCAAATACTA CTTCAAGGAT TTGCCATGT CTCCACGGGG CGTCATCGCA TCAAATTTAC 780
ACTTCAGCTG GGACGGTTTC ACGAGAGATG CTTGCAGGA TTTGTTGACA AAGTACTTCA 840
AACTTGCCAG ATGTGATTGG AAGAATACGG TTGGCAAGTT TCAAATCTTC CATCAGGCAG 900
CGGAAGAGTT TGTCATGTAC TTGTATACAT CTTACTCGAA CGACGCCGAG CGCGAAGTTG 960
CCCAAGACCG TCACTATCAT TTGGAGGCTG ACATAGAACA GATCTACAAA ACATGCGAGC 1020
CCACCAAAGC GCTTGGCGGG CATGCTGGGT GGGCGCCGTT CCCCCTGCGG CCGCGCAAGA 1080

GGCACACATC CAACACGTCG TATATGCATG ACGAGACGAT GGACTIONCCCC TTCTACGCGC 1140
 TCACTGAGAC GATCAACGGC TCCGGGCGGA ATCAGCGCGG CAAGTACAAG TCTGCGTACA 1200
 TGATCAAGGA TTTCOCGGAT TTCCAGATCG ACGTGATCTG GAAATACCTT ACGGAGGTCC 1260
 CGGACGGCTT GACTAGTGCC GAAATGAAGG ATGCCTTACT CCAGGTGGAC ATGTTTGGTG 1320
 GTGAGATTCA CAAGGTGGTC TGGGATGCGA CGGCAGTCGC GCAGCGCGAG TACATCATCA 1380
 AACTGCAGTA CCAGACATAC TGGCAGGAAG AAGACAAGGA TGCAGTGAAC CTCAAGTGGA 1440
 TTAGAGACTT TTACGAGGAG ATGTATGAGC CGTATGGCGG GGTTCAGAC CCCAACACGC 1500
 AGGTGGAGAG TGGTAAAGGT GTGTTTGAGG GATGCTACTT CAACTACCCG GATGTGGACT 1560
 TGAACAACTG GAAGAACGGC AAGTATGGTG CCCTCGAACT TTACTTTTIG GGTAACTGA 1620
 ACCGCCTCAT CAAGGCCAAA TGGTTGTGGG ATCCCAACGA GATCTTCACA AACAAACAGA 1680
 GCATCCCTAC TAAACCTCTT AAGGAGCCCA AGCAGACGAA ATAGTAGGTC ACAATTAGTC 1740
 ATCGACTGAA GTGCAGCACT TGTCGGATAC GGCCTGATGG TTGCTTTTITA TAAACTTGGT 1800
 A 1801

29. A microbial cell which comprises the recombinant DNA molecule of claims 26.

30. A cell according to claim 29 which is selected from the group consisting of a bacterial cell, a fungal cell and a yeast cell.

31. A cell according to claim 30 which is selected from the group consisting of an *E. coli* cell, a lactic acid bacterial cell, a *Saccharomyces cerevisiae* cell and a *Pichia pastoris* cell.

10 32. A method of manufacturing a food product wherein a polypeptide according to claim 9 or a microbial cell according to claim 29 is used.

33. A method according to claim 32 wherein the food product is selected from the group consisting of a dairy product, a starch-containing food product and a non-dairy beverage.

FOOTNOTES

35. A method according to claim 32 wherein the polypeptide is acting as an oxygen removing agent in a food packaging.

36. A method of manufacturing an animal feed wherein the polypeptide according to claim 9 or a microbial cell according to claim 29 is used.

37. A method according to claim 36 wherein the animal feed is
10 silage.

38. A method of reducing the sugar content of a food product, comprising adding to said product an amount of the polypeptide according to claims 9 or a microbial cell according to claim 29 which is sufficient to remove at least part of the sugar initially present in said food product.

39. A method of manufacturing a product selected from the group consisting of a pharmaceutical product, a cosmetic and a tooth care product wherein a polypeptide according to claim 9 or a microbial cell according to claim 29 is used.

40. A method of preparing a baked product from a dough, comprising adding the polypeptide according to claim 9 or a microorganism according to claims 29 capable of expressing such a polypeptide to the dough.

25 41. A dough improving composition comprising a polypeptide
according to claim 9 or a microorganism according to claim 29
capable of expressing such a polypeptide in dough, and at
least one conventional dough component.

42. A composition according to claim 41, further comprising
30 at least one enzyme selected from the group consisting of a
cellulase, a hemicellulase, a xylanase, a pentosanase, an
amylase, a lipase and a protease.

43. A method of analyzing the content of a sugar in a sample wherein the polypeptide according to claim 9 or the microbial cell according to claims 29 is used as an analytical reagent.

44. A method of manufacturing a lactone using a polypeptide according to claim 9 or a microbial cell according to claims 29, said method comprising applying the polypeptide and/or the microbial cell to a reactor containing a carbohydrate which can be oxidized by the polypeptide and operating the reactor under conditions where the carbohydrate is oxidized to a lactone.

TECHNOLOGICAL